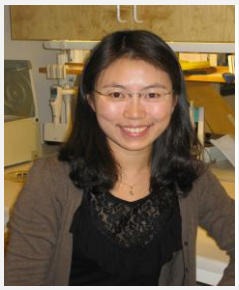
# ImmunoTools *special* Award 2014



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## Investigation of the role of microRNAs in skin wound healing

Wound healing is a fundamental physiological process to keep integrity of the skin; fails of healing result in chronic wound, which is trapped in a constant inflammatory state and the differentiation and activation of keratinocytes are deregulated. Chronic wound is a common medical problem with high morbidity and mortality, but its molecular pathogenesis remains poorly understood which impedes the development of effective treatment. MicroRNAs are ~22nt noncoding RNAs, which are gene regulators essential for multiple biological functions in the skin and represent important targets for potential therapeutic agents; however, their role in wound healing remains largely unknown.

**The objective of our study is to reveal the role of miRNAs in skin wound healing** and **to explore the potential of miRNA-based therapy for chronic wounds.** For this, we will first characterize miRNA expression profiles (miRNAome) of injured human skin from healthy donors at different phases (inflammation, proliferation and remodelling) of wound healing and compare them with miRNAome of chronic wounds. Next, we will study the regulation and function of differentially expressed miRNAs in skin cells by state-of-the art *in vitro* experiments. To further understand the function of miRNAs, we will identify the target genes regulated by these wound healing related miRNAs. Moreover, we will investigate the *in vivo* role and the therapeutic potential of miRNAs for wounds using human *ex vivo* and mouse *in vivo* wound models. This study will increase our understanding about the role of miRNAs in skin biology and their target genes with possible implications in pathogenesis of chronic wounds, which will provide a basis for developing novel miRNA-based therapy.

We have analyzed miRNAome in inflammation phase of human wound healing and

identified 55 miRNAs differentially expressed compared to intact skin. Moreover, we found one of the top-regulated miRNAs can regulate the production of inflammatory cytokines/chemokines expression by keratinocytes, which are important for attracting immune cells to wounds. Our current study focus on investigation of the cross-talk between keratinocytes and immune cells in normal and chronic wounds.

The reagents from ImmunoTools will majorly contribute to our project studying the mechanisms regulating miRNA expression during wound healing. As a novel regulator, the expression of miRNA itself is also subjected to complicated regulation. Studying the mechanisms regulating miRNA expression will, from another angle, increase our understanding about normal skin wound healing and the pathogenesis of chronic wounds. For this, we will initially focus on keratinocytes, one of the major players in skin wound healing process. We will treat human primary keratinocytes with different factors important for skin wounds, including scratch-injury, cytokines (e.g. IL-1, IL-6, IL-22, TNF-α, TGF-β), growth factors (e.g. EGF, FGF, IGF, GM-CSF, KGF), and toll-like receptor (TLR) ligands [e.g. zymosan, poly (I:C), flagellin]. The RNA will be extracted from the treated cells and miRNA expression will be analyzed with real time PCR (to measure specific miRNA expression) or TaqMan Low Density Array (to analyze miRNAome), which may reveal the regulation mechanisms of some differentially expressed miRNAs during wound healing process. Furthermore, we aim to dissect the molecular pathways and identify transcription factors, which may be responsible for the regulation of miRNAs in wounds. The promoter region of the miRNA of interest will be cloned into a luciferase reporter vector and promoter luciferase assay will be performed in the cells treated with pathologically relevant factors identified above. The role of individual transcription factors will be defined by site-specific mutagenesis of their binding sites.